Decreased Hepatic Expression of the Low-Density Lipoprotein (LDL) Receptor and LDL Receptor–Related Protein in Aging Rats Is Associated With Delayed Clearance of Chylomicrons From the Circulation

Polly A. Field and Geoffrey F. Gibbons

Aging in both humans and rats is associated with the development of insulin resistance and the ensuing alterations in the plasma lipoprotein profile. In this study, young (2 months) and old (15 months) Sprague-Dawley (SD) rats were used to investigate age-related alterations in the chylomicron clearance pathway. Clearance from the blood of an intravenously injected bolus of ^{14}C-labeled cholesterol ester (CE) and ^{3}H-labeled triacylglycerol (TAG) lymph chylomicrons was markedly delayed in the old rats ($P < .05$). Hepatic expression of the two principal receptors of chylomicron remnant removal, the low-density lipoprotein (LDL) receptor and LDL receptor–related protein (LRP), was determined by ligand blotting and immunoblotting. The old rats expressed 43% ± 7% of the level of LDL receptor in the young animals ($P < .05$) and 45% ± 16% of the corresponding level of LRP ($P < .05$). The results suggest that the delayed clearance of chylomicron remnants in this animal model of aging and insulin resistance is due, at least in part, to a decrease in the hepatic expression of LDL receptor and LRP.

Copyright © 2000 by W.B. Saunders Company

THE DEVELOPMENT of insulin resistance is believed to be a natural consequence of aging and the alterations in body composition, specifically an increase in the mass of abdominal adipose tissue, entailed therein.[1-3] Insulin resistance is characterized by several perturbations in the plasma lipoprotein profile including postprandial lipemia,[4] which is thought to be a central feature in the etiology of atherosclerosis.[5-8] Chylomicrons and very–low-density lipoprotein (VLDL) are cleared by a common 2-step clearance pathway, which may be altered at several potential loci by insulin resistance. Upon entry to the bloodstream, chylomicrons react with lipoprotein lipase (LPL) on the capillary endothelium to release a proportion of their component triacylglycerol (TAG) as nonesterified fatty acids (NEFAs).[9,10] The TAG-depleted chylomicrons (known as chylomicron remnants) are then cleared from the circulation, predominantly by the low-density lipoprotein (LDL) receptor and LDL receptor–related protein expressed by the liver (reviewed recently[11,12]). It has been established that chylomicron clearance is delayed in elderly humans.[13-15] A number of separate studies, including LDL kinetic studies in man[16] and studies of LDL binding to canine liver cell membranes,[17,18] have provided indirect evidence of a decline in hepatic expression of the LDL receptor with progressive aging. Until now, extension of this hypothesis to humans or rats has awaited direct determination of receptor expression in these species. The influence of age on the hepatic expression of LRP in any species has yet to be addressed.

In the present investigation, Sprague-Dawley (SD) rats were studied. The aging SD rat exhibits several of the main metabolic features of human aging,[1,19] including increased plasma insulin and NEFAs and increased adiposity, especially visceral fat.[20,21] The insulin resistance of the old animals is manifest both peripherally and hepatically.[21-24] A comparison between the rates of peripheral lipolysis and hepatic removal of chylomicrons between young and old rats was achieved by injecting lymph chylomicrons radiolabeled in the TAG and cholesterol ester (CE) moieties. The fact that CE is not hydrolyzed by LPL but remains with the particle enables it to serve as a marker of particles remaining in the blood prior to hepatic uptake. Hepatic expression of the LDL receptor and LRP was directly determined by immunoblotting and ligand blotting. It was established that the rate of chylomicron remnant clearance and the level of expression of the LDL receptor and LRP were reduced in the old rats in comparison to the younger animals.

From the Metabolic Research Laboratory, Oxford Lipid Metabolism Group, University of Oxford, Oxford, UK.

Submitted June 2, 1999; accepted September 2, 1999.

Supported by a Medical Research Council (MRC) research studentship (to P.A.F.) and a MRC program grant (to G.F.G.).

Address reprint requests to Geoffrey F. Gibbons, PhD, Metabolic Research Laboratory, Radcliffe Infirmary, Woodstock Road, Oxford, OX2 6HE.

Copyright © 2000 by W.B. Saunders Company

0026-0495/00/4904-0016$10.00/0

MATERIALS AND METHODS

Animals

Male SD rats aged either 2 months or 15 months (designated young and old, respectively) were used for the chylomicron clearance kinetics and receptor expression studies. Chylomicrons were harvested from adult male Wistar rats (150 to 200 g body weight). All animals were supplied by Harlan Olac, Bicester, UK and were housed at 22° ± 2°C in a windowless room artificially lit between 8 AM and 8 PM. Animals were allowed ad libitum access to tap water and a pelleted diet (4.3% fat, 22.3% protein, 4.6% fiber, and 51.2% carbohydrate [mainly starch]; supplied by Special Diet Services, Witham, Essex, UK). All procedures were performed between 9 and 11 AM.

Chylomicron Clearance Kinetic Studies

Radiolabeled lymph chylomicrons were prepared by cannulation of the thoracic lymph duct of a rat anesthetized with halothane.[25] An aliquot of triolein (1 mL) containing [9,10(n)-^{3}H]oleic acid (14.8 MBq/mL) and [4-^{14}C]cholesterol (1.67 MBq/mL) was introduced directly into the stomach. The lymph was collected into a flask containing EDTA, reduced glutathione, and gentamycin sulfate to a final concentration of 2.7 mmol/L, 1.6 mmol/L, and 0.1 mg/mL, respectively. The resultant chylomicron suspension was filtered through nylon gauze and centrifuged at 120,000 × g for 35 minutes at 10°C. The surface layer of chylomicrons was mixed with 0.9% (wt/vol) sterile saline and centrifuged again under the same conditions. The chylomicron suspension was aspirated from the surface. Penicillin (100 U/mL) and streptomycin (0.1 mg/mL) was added and the solution was stored at 4°C away from light until use. Only one preparation of chylomicrons was used for the studies described in this report.

Recipient animals were anesthetized with pentobarbital injected intraperitoneally (65 mg/kg body weight). A heparinized blood sample was taken from the tail before administration of the chylomicron suspension (30 mg chylomicron-TAG/kg body weight) via the saphenous vein. At set time points following chylomicron administration, heparinized blood samples were taken from the tail and kept on ice. After 30 minutes, the rat was bled by aortic puncture and the liver was removed, quickly perfused with a solution containing 7.1 g/L sodium chloride, 0.36 g/L potassium chloride, 0.165 g/L potassium dihydrogen phosphate, 0.302 g/L magnesium sulfate heptahydrate, and 2.15 g/L sodium hydrogen carbonate, blotted, and frozen in liquid nitrogen. The blood samples taken throughout the time course were centrifuged for 10 minutes (700 × *g* at 4°C) and the plasma was aspirated.

Analytical Procedures

Immediately after the experiment, lipids were extracted from the plasma samples by the method of Folch et al[26] and fractionated by thin-layer chromatography (TLC)[27] (Silica gel G plates; Anachem, Bedfordshire, UK). The TAG and CE bands were scraped from the plate and radioactivity was determined by scintillation counting (Optiphase 'Safe' scintillation fluid, Wallac, Milton Keynes, UK; and LS 6500 Beckman scintillation counter, High Wycombe, UK). The initial and terminal blood samples were analyzed for total TAG, NEFA, and glucose using a Monarch Clinical Chemistry analyzer (Instrumentation Laboratories, Warrington, UK). The plasma glucose level was measured by the hexokinase method, plasma NEFA was assayed using a WAKO NEFA C kit (Alpha Laboratories, Eastleigh, UK), and plasma TAG was determined according to Humphreys et al.[28] Liver samples were homogenized in phosphate-buffered saline ([PBS] 1:5 liver to buffer) using a top-drive Potter-Elvehjem homogenizer (Voss Instruments, Maldon, Essex, UK). Lipids were extracted from an aliquot of homogenate by the method of Folch et al,[26] separated by TLC, and assayed for radioactivity as before.

Preparation of Liver Plasma Membrane Fraction

Rats were killed by concussion followed by dislocation of the neck. Liver homogenates were prepared in 50 mmol/L HEPES (pH 7.4), 100 mmol/L NaCl, 10 mmol/L EDTA, 10 mmol/L EGTA, 10 mmol/L *n*-ethylmaleimide, 2.2% (vol/vol) dimethyl sulfoxide, and 1 mmol/L PMSF using a top-drive Potter-Elvehjem homogenizer. The homogenates were sequentially centrifuged (at 4°C) first at 500 × *g* for 5 minutes, followed by 8,000 × *g* for 1 hour, and finally 100,000 × *g* for 1 hour to pellet the membrane fraction (referred to as plasma membrane) as described by Kovanen et al.[29] The pellets were combined and sonicated in the same buffer until the suspension was homogenous (Vibracell sonicator with 0.2-cm probe; Sonics and Materials, Danbury, CT). The suspension was stored in liquid nitrogen till use.

Immunoblotting

Proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using 5% acrylamide minigels under nonreducing conditions. Fractionated proteins were electroblotted onto nitrocellulose membrane (Hybond ECL; Amersham, Buckinghamshire, UK). Nitrocellulose membranes were blocked for 1 hour in a solution of PBS-Tween, pH 7.5 (80 mmol/L) disodium hydrogen orthophosphate, 20 mmol/L sodium dihydrogen orthophosphate, 100 mmol/L sodium chloride, and 0.1% vol/vol Tween 20) containing 5% (wt/vol) nonfat milk powder. The nitrocellulose membrane was then incubated for 1 hour with either an antibody to the LDL receptor diluted 1:1,000 in PBS-Tween[30] or an antibody against the 85-kd subunit of LRP diluted 1:2,000 in PBS-Tween.[31] Nitrocellulose membranes were washed in PBS-Tween (3 changes of buffer over 20 minutes) and incubated for 1 hour with a secondary antibody, rabbit immunoglobulin horseradish peroxidase–linked whole antibody (Amersham), diluted 1:1,000 in PBS-Tween. Nitrocellulose membranes were washed as before, and the bands were detected by enhanced chemiluminescence ([ECL] Amersham ECL reagents and Hyperfilm-ECL). Relative band intensities were determined by densitometry (BioRad, Hemel Hempstead, UK).

Ligand Blotting

Proteins were fractionated and electroblotted as described before. The nitrocellulose membranes were incubated with a blocking buffer for 1 hour at 37°C (50 mmol/L Tris/hydrochloride, pH 8.0, 90 mmol/L sodium chloride, 2 mmol/L calcium chloride dihydrate, and 50 mg/mL albumin bovine fraction V). An aliquot of rabbit βVLDL (isolated as previously described[32]) and iodinated by the iodine monochloride method[33] to a specific activity of 672 cpm/ng) was suspended in blocking buffer to a final concentration of 1 μg protein/mL and incubated with the membrane for 1 hour. Unbound ligand was removed by washing in a buffer identical to the blocking buffer but containing only 5 mg/mL albumin. Ligand binding was detected by autoradiography using preflashed film (Hyperfilm-ECL) and enhancer screens, and the relative band intensities were determined by densitometry (BioRad Model GS-700 Imaging Densitomiter).

Calculations

The total plasma volume for SD rats was calculated from the following formula[34]: plasma volume = 0.175 (weight$^{0.725}$). The proportion of label remaining in the plasma against time was plotted for each rat, and the area under the clearance curve (AUC) was calculated. The AUCs between different groups of rats were compared using the Mann-Whitney *U* test for 2 independent samples to determine statistically significant differences. To indicate the lipolytic processing of chylomicrons throughout the time course, the removal of ^{3}H TAG in excess of ^{14}C CE at each time point was calculated (the proportion of ^{3}H TAG removed minus the proportion of ^{14}C CE removed). The Mann-Whitney *U* test for 2 independent samples was used to determine statistically significant differences. Plasma membrane samples from 4 young and 4 old rats were separated on the same acrylamide gel (equal amounts of protein applied), and the intensity of the LDL receptor or LRP (in arbitrary units) was determined by scanning laser densitometry. Significant differences between young and old rats were determined by Mann-Whitney *U* test for 2 independent samples. All values are expressed as the mean ± SE, and all statistical calculations were performed using SPSS Version 8 for Windows (SPSS, Chicago, IL).

RESULTS

Chylomicron Clearance Kinetics

A bolus of radiolabeled lymph chylomicrons was injected into 3 old rats (body weight, 518 ± 23 g) and 3 young rats (body weight, 232 ± 5.0 g). The clearance of chylomicron remnants from the plasma (indicated by loss of ^{14}C CE) was markedly slower in old rats than in younger animals ($P < .05$; Fig 1). Thirty minutes after the injection, almost twice the proportion of ^{14}C CE remaining in the blood of young rats was retained in the blood of older animals (11.15% ± 1.9% *v* 19.52% ± 4.3% of the injected dose). Similarly, the clearance of ^{3}H TAG was retarded in old rats ($P < .05$). However, by 30 minutes, the relative proportions of ^{3}H TAG remaining in the plasma of young and old animals were not greatly different: 1.78% ± 0.2% of the injected TAG remained in young rats *v* 2.94% ± 0.65% in old rats. A comparison of total plasma TAG in samples taken before administration of the chylomicrons and 30 minutes later indicated that old rats but not young rats

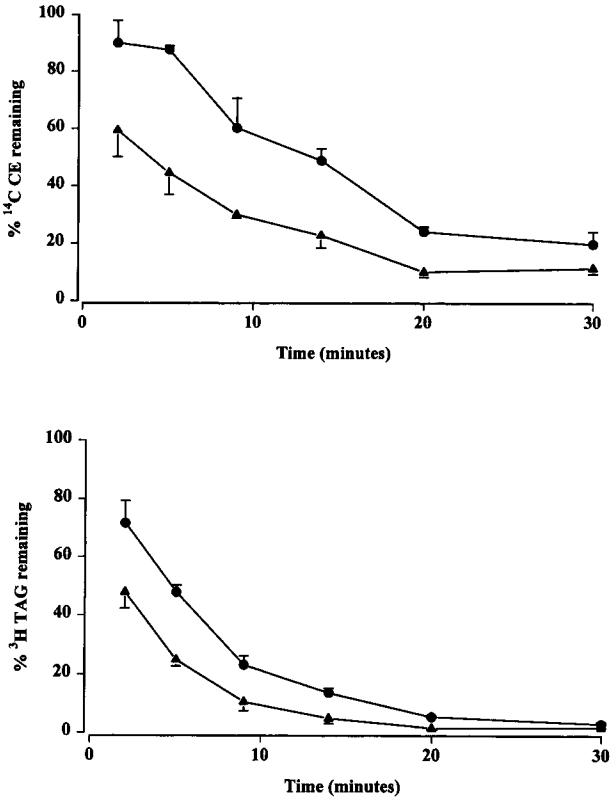

Fig 1. Decay of chylomicron label within the blood. Clearance of ^{14}C CE and ^{3}H TAG from the plasma following chylomicron administration to 3 old rats (●) and 3 young rats (▲). Clearance of ^{14}C CE and ^{3}H TAG was significantly slower in old rats ($P < .05$).

significantly accumulated TAG over the experimental period ($P < .05$) (Table 1).

To help indicate the degree of chylomicron TAG removed in the circulation throughout the time course, the excess clearance of ^{3}H TAG to ^{14}C CE at each time point was calculated from the data in Fig 1 (Fig 2). The premise for the calculation was the assumption that removal of chylomicrons from the blood as intact particles would entail an equal disappearance of ^{3}H TAG from the blood as ^{14}C CE removal. Because chylomicrons are not cleared from the blood without the loss of some ^{3}H TAG, the excess clearance of ^{3}H TAG is a reflection of that proportion of chylomicron ^{3}H TAG which has been removed by peripheral lipolysis. Therefore, subtracting the proportion of ^{14}C CE which has been removed from the blood at each time point from the proportion of ^{3}H TAG removed provides an indication of the

Table 1. Analysis of Plasma Before Chylomicron Injection and 30 Minutes Afterward

Group	Glucose (mmol/L)	NEFA (μmol/L)	TAG (μmol/L)
Before			
Old	5.69 ± 0.34[a]	425 ± 28[b]	529 ± 70[c]
Young	5.76 ± 0.18	253 ± 9[b]	380 ± 64
After			
Old	7.09 ± 0.4[a]	331 ± 91	831 ± 17[c]
Young	6.53 ± 0.42	249 ± 17	484 ± 80

NOTE. Values that are significantly different are denoted by the same superscript ($P < .05$).

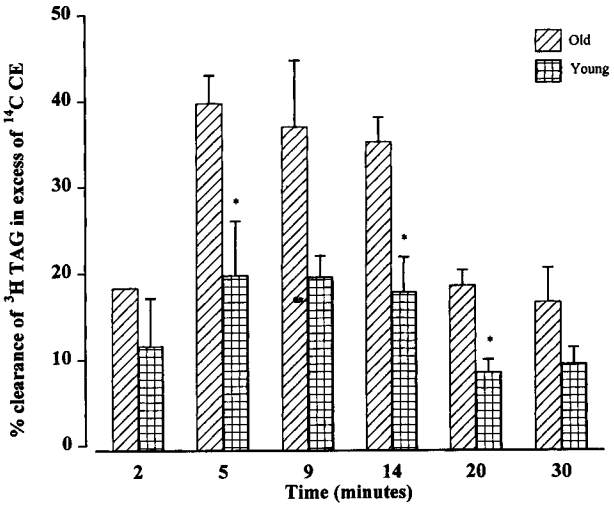

Fig 2. Lipolytic loss of chylomicron TAG throughout the time course, calculated from the proportion of ^{3}H TAG cleared in excess of the clearance of ^{14}C CE. *$P < .05$, young *v* old rats.

proportion of ^{3}H TAG which has been cleared from the circulation by peripheral lipolysis. Figure 2 demonstrates that a greater proportion of chylomicron TAG was cleared from the circulation following peripheral lipolysis in the old rats versus the younger animals ($P < .05$ at 5, 14, and 20 minutes).

Hepatic Clearance

Analysis of the liver samples taken after the 30-minute time course showed no significant differences between the recovery of chylomicron ^{3}H TAG or ^{14}C CE between the young and old animals. The old rats incorporated 59% ± 1.1% of the injected chylomicron ^{14}C CE, whereas the young rats incorporated 62% ± 4%; 26.7% ± 1.1% of the injected ^{3}H TAG was recovered in old rats versus 23.4% ± 2.5% in the younger animals.

Hepatic Receptor Studies

The 4 old and 4 young rats used for this study were in all respects identical to those used for the chylomicron kinetic study. The determination of the relative hepatic expression of LDL receptor by ligand blotting showed that old rats expressed less than half the level of the receptor of the younger rats ($P < .05$; Table 2 and Fig 3). This finding was confirmed by the immunoblotting data (Fig 4). The antibody raised against the 85-kd subunit of LRP bound to an 85-kd protein and, to a lesser extent, to a 170-kd protein (approximately 25% of the binding to 85kd; Fig 5). The higher–molecular weight band was presumably a dimer of the 85-kd subunit. A decreased expression of LRP in the old rats was evident from analysis of either

Table 2. Relative Hepatic Expression of LDL Receptor and LRP as Quantified by Laser Scanning Densitometry (arbitrary units)

	Old	Young
Ligand blot of LDL receptor	43.0 ± 7.3*	100 ± 14.5
Immunoblot of LDL receptor	58.0 ± 22.5	100 ± 11.3
Immunoblot of LRP	45.0 ± 15.9*	100 ± 1.2

*$P < .05$, young *v* old rats.

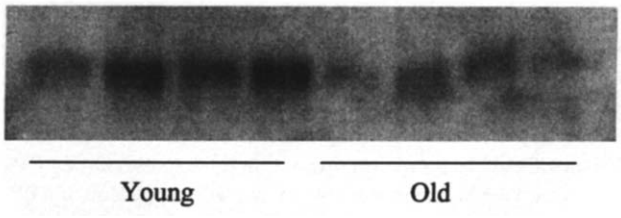

Fig 3. Ligand blot of the LDL receptor. Hepatic plasma membrane fractions from 4 young and 4 old rats were subjected to SDS-PAGE, electroblotted onto nitrocellulose, and incubated with ^{125}I-βVLDL. Bands were visualized by autoradiography.

the 85-kd subunit (45% ± 15.9% of the expression of the young animals) or its dimer (52% ± 3.5% of the expression of the young animals, $P < .05$).

DISCUSSION

These results provide direct evidence for an impeded clearance of chylomicrons from the plasma of old SD rats. However, since the clearance of TAG and CE moieties of the chylomicrons was delayed in the old rats, it was not immediately apparent whether peripheral lipolysis or whole-particle uptake was "rate-limiting" to particle clearance. Distinguishing between an accumulation of chylomicrons owing to a defect of lipolysis and an accumulation caused by insufficient hepatic clearance poses a considerable experimental challenge. Indeed, to address this question fully, a separation of the lipolytic and hepatic steps would be necessary. Nevertheless, a comparison of the clearance of chylomicron TAG to the clearance of CE from the rats in this study can help in making this distinction. Following the injection of chylomicrons into old rats, only a small proportion of ^{14}C CE was removed from the blood between 2 and 5 minutes (90% ± 7.8% to 88% ± 1.3% of the injected dose remaining in the blood). Only after 9 minutes did

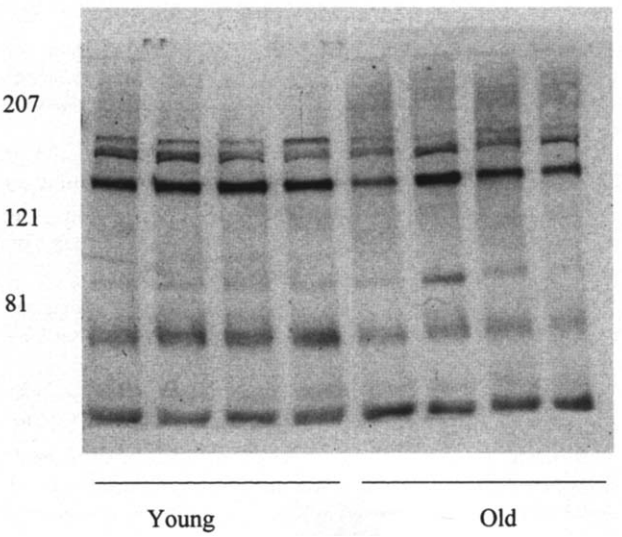

Fig 4. Immunoblot of the LDL receptor. Hepatic plasma membrane fractions from 4 young and 4 old rats were subjected to SDS-PAGE, electroblotted onto nitrocellulose, and incubated with a polyclonal antibody against the LDL receptor. Bands were visualized by ECL. Molecular weight markers (in kd) are indicated.

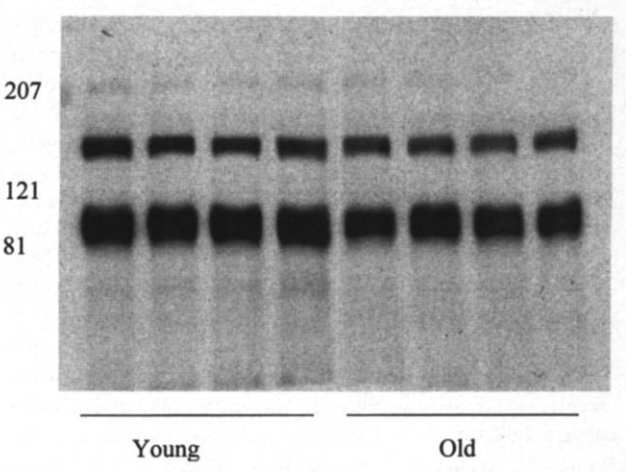

Fig 5. Immunoblot of the LRP. Hepatic plasma membrane fractions from 4 young and 4 old rats were subjected to SDS-PAGE, electroblotted onto nitrocellulose, and incubated with a polyclonal antibody against the 85-kd subunit of LRP. Bands were visualized by ECL. Molecular weight markers (in kd) are indicated.

the level of ^{14}C CE decrease substantially. The clearance of ^{3}H TAG showed a different pattern. There was a rapid removal of TAG between 2 and 5 minutes (72% ± 7.6% to 48% ± 2.4% of the injected dose remaining in the blood). It is therefore apparent that chylomicrons were subject to lipolysis during the first 9-minute period yet hepatic clearance was minimal. Lipolytic removal of TAG from a chylomicron is essential for subsequent hepatic removal.[9,35] To establish whether the degree of lipolysis impaired hepatic removal in old rats, a comparison to the young rats was made. Figure 2 demonstrates that the proportional loss of TAG from the blood as a result of lipolysis was higher in the old rats. It is therefore evident that the loss of TAG in old rats would be sufficient to allow hepatic internalization if the liver possessed that capacity. Because whole-particle uptake from the plasma was restricted in the old rats, chylomicrons were exposed for a longer period to lipolytic TAG removal, resulting in a proportionally higher clearance of TAG from the blood via lipolysis. This hypothesis accords with findings by Summerfield et al,[36] who demonstrated that the activity of LPL was not altered between the ages of 3 and 13 months in SD rats. The lower rate of clearance of chylomicron TAG is therefore thought to be secondary to a reduced rate of removal of whole particles from the blood. The finding that the hepatic recovery of radiolabel 30 minutes following chylomicron administration was not significantly different between the old and young rats does not contradict this conclusion. By 30 minutes, the slower clearance mechanisms of the old rats achieved a total clearance similar to that found in the younger animals; absolute clearance was delayed, not significantly reduced.

A reduced rate of lipoprotein clearance in the old rats was further indicated by the increased plasma levels of total TAG in old animals following the 30-minute experimental period (Table 1). The extra TAG cannot simply be attributed to residual chylomicrons, as the 3% of the injected chylomicron TAG remaining in the plasma after 30 minutes would increase the level of TAG by approximately 30 μmol/L. It is more likely that

the elevated TAG was a consequence of a larger number and/or larger size of VLDL in the plasma: a preferential clearance of chylomicrons over VLDL via the common clearance pathway resulting in an accumulation of endogenous lipid in the plasma.[7,37,38] The immunoblotting and ligand blotting of hepatic membranes provides a mechanistic explanation for the reduced remnant clearance of the old rats by demonstrating very clearly that expression levels of both the LDL receptor and LRP were decreased. The old rats showed a level of expression of both receptors, per unit weight of liver, that was only half of that in the younger animals (Table 2). Why the expression levels of LDL receptor and LRP were decreased in old rats is not immediately obvious. The principal means of regulating LDL receptor expression is through alterations in cellular sterol balance. Influx of cholesterol to the cell, via endocytic internalization of lipoproteins, results in a decline of cholesterol synthesis and LDL receptor expression.[39-41] It is plausible that the insulin resistance manifest in the old rats may have altered the cholesterol balance of the liver and thus affected the regulation of LDL receptor. Studies in man lend tentative support to this hypothesis. The ability of LDL isolated from type 2 diabetic subjects to decrease cholesterol synthesis is diminished.[42] Other defects in cholesterol metabolism in type 2 diabetic patients include increased levels of 3-hydroxy-3-methylglutaryl-coenzyme A (CoA) reductase,[43] reduced activity of acyl CoA:cholesterol-*O*-acyltransferase,[44] and a failure to downregulate peripheral cholesterol synthesis in the postprandial period.[45] Although the effects of these many alterations on hepatic expression of the LDL receptor have not been investigated, the increased levels of cellular free cholesterol would be anticipated to downregulate receptor expression.[46] Whether similar alterations in cholesterol metabolism exist in the insulin-resistant nondiabetic state remains to be resolved. Previous study has shown that the level of total hepatic cholesterol increases with age in the SD rat, mainly as an increase in esterified cholesterol,[47] but the size of the regulatory pool of free cholesterol in old and young rats is not known. However, the inability of the liver to respond appropriately to insulin in the insulin-resistant state may directly reduce the expression of the LDL receptor. Studies in hepatocyte culture have shown that insulin exerts a stimulatory influence on receptor expression and is able to overcome the suppressive effect of exogenous LDL.[48] The altered levels of glucagon[49,50] and thyroid hormone[51] in old rats may also have a modulating effect.[52-54]

The promoter region of the LRP gene does not contain a sterol response element.[55] Alterations in cellular cholesterol balance therefore would not impact on the expression of LRP. However, whether insulin is able to increase expression of LRP in the liver, analogous to the effect on the LDL receptor, is unknown. Evidence from adipocytes, which exhibit increased translocation of LRP from intracellular compartments to the plasma membrane when stimulated by insulin, indicates that LRP action rather than expression is insulin-sensitive.[56] The paucity of information concerning LRP regulation effectively precludes speculation on the likely regulatory mediators of LRP in insulin resistance.

It could be argued that reduced hepatic expression of endocytic receptors was secondary to a general senescence of the old rats rather than a direct consequence of insulin resistance. The results from the present study cannot distinguish between these possibilities; however, investigations of LDL receptor expression in man have indicated that aging per se does not diminish the ability of the liver to express the LDL receptor. It is known that elderly men receiving pharmacological doses of estrogen have a dramatically elevated apolipoprotein B LDL fractional catabolic rate, suggesting a capacity for upregulation of the LDL receptor.[57] Furthermore, skin fibroblasts isolated from individuals of varying ages do not show a reduced expression of the LDL receptor with age.[58]

In summary, the delayed clearance of chylomicrons from the plasma of old rats is associated with a reduced hepatic expression of the LDL receptor and LRP. Further investigation into the mechanisms by which receptor expression is regulated would increase our understanding of this important facet of aging.

ACKNOWLEDGMENT

We are indebted to several people, most importantly R. Hems for preparing the chylomicrons, B. Knight and D. Norman for the kind gift of ^{125}I-labeled βVLDL, and P. Roach and J. Herz for the respective gifts of the LDL receptor antibody and LRP antibody.

REFERENCES

1. Fraze E, Chlou M, Chen Y, et al: Age related changes in post prandial plasma glucose, insulin, and FFA concentrations in non-diabetic individuals. J Am Geriatr Soc 35:224-228, 1987

2. Coon PJ, Rogus EM, Drinkwater D, et al: Role of fat distribution in the decline of insulin sensitivity and glucose tolerance with age. J Clin Endocrinol Metab 75:1125-1132, 1992

3. Hohrt WM, Kirwan JP, Staten MA, et al: Insulin resistance in aging is related to abdominal obesity. Diabetes 42:273-281, 1993

4. Taskinen M, Lahdenpera S, Syvanne M: New insights into lipid metabolism in non insulin dependent diabetes mellitus. Ann Med 28:335-360, 1996

5. Zilversmit D: Atherogenesis: A postprandial phenomenon. Circulation 60:473-485, 1979

6. Patsch J, Miesenbock G, Hoperwisser T, et al: The relationship of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. Arterioscler Thromb 12:1336-1345, 1992

7. Karpe F, Steiner G, Olivecrona T, et al: Metabolism of triglyceride rich lipoproteins during alimentary lipaemia. J Clin Invest 91:748-758, 1993

8. Mamo J, Proctor S, Smith D: Retention of chylomicron remnants by arterial tissue; importance of an efficient clearance mechanism from plasma. Atherosclerosis 141:S63-S69, 1998 (suppl)

9. Redgrave T: Formation of cholesterol ester rich particulate lipid during metabolism of chylomicrons. J Clin Invest 49:465-471, 1970

10. Karpe F, Olivecrona T, Hamsten A, et al: Chylomicron/chylomicron remnant turnover in humans: Evidence for margination of chylomicrons and poor conversion of larger to smaller chylomicron remnants. J Lipid Res 38:949-961, 1997

11. Cooper A: Hepatic uptake of chylomicron remnants. J Lipid Res 38:2173-2192, 1997

12. Mahley R, Ji Z: Remnant lipoprotein metabolism: Key pathways

involving cell-surface heparin sulfate proteoglycans and apolipoprotein E. J Lipid Res 40:1-16, 1999

13. Cohn JS, McNamara JR, Cohn SD, et al: Post-prandial plasma lipoprotein changes in human subjects of different ages. J Lipid Res 29:469-479, 1988

14. Krasinski SD, Cohn JS, Schaefer EJ, et al: Postprandial plasma retinyl ester response is greater in older subjects compared with younger subjects. J Clin Invest 85:883-892, 1990

15. Borel P, Mekki N, Boirie Y, et al: Comparison of the postprandial plasma vitamin A response in young and older adult. J Gerontol A Biol Sci Med Sci 53:B133-B140, 1998

16. Ericsson S, Eriksson M, Vitols S, et al: Influence of age on the metabolism of plasma low density lipoproteins in healthy males. J Clin Invest 87:591-596, 1991

17. Mahley RW, Innerarity TL: Lipoprotein receptors and cholesterol homeostasis. Biochim Biophys Acta 737:197-222, 1983

18. Mahley RW, Hui DY, Innerarity TL, et al: Two independent lipoprotein receptors on hepatic membranes of the dog, swine and man: Apo-B,E and apo-E receptors. J Clin Invest 68:1197-1206, 1981

19. Reaven G: Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988

20. Barzilai N, Rossetti L: The relationship between changes in body composition and insulin responsiveness in models of aging rats. Am J Physiol 269:E591-E597, 1995

21. Barzilai N, Rossetti L: Age-related changes in body composition are associated with hepatic insulin resistance in conscious rats. Am J Physiol 270:E930-E936, 1996

22. Goodman MN, Dluz SM, McElaney MA, et al: Glucose uptake and insulin sensitivity in rat muscle: Changes during 3-96 weeks of age. Am J Physiol 244:E93-E100, 1983

23. Narimiya M, Azhar S, Dolkas CB, et al: Insulin resistance in older rats. Am J Physiol 246:E397-E404, 1984

24. Nishimura H, Kuzuya H, Okamoto M, et al: Change in insulin action with aging in conscious rats determined by euglycaemic clamp. Am J Physiol 254:E92-E98, 1988

25. Bollman J, Cain J, Grindlay J: Techniques for the collection of lymph from the liver, small intestine, or thoracic duct of the rat. J Lab Clin Med 33:1348-1352, 1948

26. Folch J, Lees M, Sloane Stanley GH: A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497-509, 1956

27. Goldfarb S, Barber TA, Pariza MW, et al: Lipid synthesis and ultrastructure of adult rat hepatocytes during their first twenty-four hours in culture. Exp Cell Res 117:39-46, 1978

28. Humphreys S, Fisher R, Frayn K: Micro method for measurement of sub-nanomolar amounts of triacylglycerol. Ann Biochem 27:597-598, 1990

29. Kovanen P, Brown M, Goldstein J: Increased binding of low density lipoprotein to liver membranes from rats treated with 17 alpha-ethinyl estradiol. J Biol Chem 254:11367-11373, 1979

30. Szanto A, Balasubramaniam S, Roach PD, et al: Modulation of the low-density-lipoprotein-receptor-related protein and its relevance to chylomicron-remnant metabolism. Biochem J 288:791-794, 1992

31. Herz J, Kowal R, Goldstein J, et al: Proteolytic processing of the 600kd low density lipoprotein receptor–related protein occurs in a trans-Golgi compartment. EMBO J 9:1769-1776, 1990

32. Wade D, Knight B, Soutar A: Binding of low density lipoprotein and chylomicron remnants to the hepatic low density lipoprotein receptor of dogs, rats and rabbits demonstrated by ligand blotting. Failure to detect a distinct chylomicron remnant binding protein by ligand blotting. Eur J Biochem 159:333-340, 1986

33. Soutar A, Knight B: Degradation by cultured monocyte-derived macrophages from normal and familial hypercholesterolaemic subjects of modified and unmodified low density lipoproteins. Biochem J 204:549-556, 1982

34. Wang C, Hegsted D: Normal blood volume, plasma volume and thiocyanate space in rats and their relation to body weight. Am J Physiol 156:218-226, 1949

35. Carrella M, Cooper A: High affinity binding of chylomicron remnants to rat liver plasma membranes. Proc Natl Acad Sci USA 76:338-342, 1979

36. Summerfield JA, Applebaum-Bowden D, Hazzard WR: Effects of diet and age on lipoprotein lipase and hepatic triglyceride lipase activities in the rat. Proc Soc Exp Biol Med 175:158-163, 1984

37. Potts J, Fisher R, Humphreys S, et al: Peripheral triacylglycerol extraction in the fasting and post-prandial states. Clin Sci (Colch) 81:621-626, 1991

38. Bjorkegren J, Packard C, Hamsten A, et al: Accumulation of large very low density lipoprotein in plasma during intravenous infusion of a chylomicron like triglyceride emulsion reflects competition for a common lipolytic pathway. J Lipid Res 37:76-86, 1996

39. Brown M, Goldstein J: Familial hypercholesterolaemia: Defective binding of lipoproteins to cultured fibroblasts associated with impaired regulation of 3-hydroxy-3-methyl-CoA reductase activity. Proc Natl Acad Sci USA 71:788-792, 1974

40. Goldstein J, Brown M, Anderson R, et al: Receptor mediated endocytosis: Concepts emerging from the LDL receptor system. Annu Rev Cell Biol 1:1-39, 1985

41. Briggs M, Yokoyama C, Wang X, et al: Nuclear protein that binds sterol regulatory element of LDL receptor promoter. I. Identification to the protein and delineation of its target nucleotide sequence. J Biol Chem 268:14490-14496, 1993

42. Owens D, Maher V, Johnson A, et al: Cellular cholesterol regulation—A defect in the type 2 (noninsulin dependent) diabetic patient in poor metabolic control. Diabetologia 33:93-99, 1990

43. Maher V, Devrey R, Collins P, et al: Relationship between low density lipoprotein cholesterol and 3 hydroxy 3 methylglutaryl coA reductase activity in mononucleocytes from type 2 diabetics. Diabetologia 30:553A, 1987 (abstr)

44. Owens D, Stinson J, Collins P, et al: Improvement in the regulation of cellular cholesterologenesis in diabetes: The effect of reduction in serum cholesterol by simvastatin. Diabet Med 8:151-156, 1991

45. Griffin M, Frazer A, Johnson A, et al: Cellular cholesterol synthesis—The relationship to post prandial glucose and insulin following weight loss. Atherosclerosis 138:313-318, 1998

46. Spady D, Wollet L, Dietschy J: Regulation of plasma LDL cholesterol levels by dietary cholesterol and fatty acids. Annu Rev Nutr 13:355-381, 1993

47. Stahlberg D, Angelin B, Einarsson K: Age related changes in the metabolism of cholesterol in rat liver microsomes. Lipids 26:349-352, 1991

48. Wade DP, Knight BL, Soutar AM: Regulation of low density lipoprotein receptor mRNA by insulin in human hepatoma Hep G2 cells. Eur J Biochem 181:727-731, 1989

49. Codina J, Vall M, Herrera E: Changes in plasma glucose, insulin and glucagon levels, glucose tolerance tests and insulin sensitivity with age in the rat. Diabete Metab 6:135-139, 1980

50. Poli G, Cassader M, Chiarpotto E, et al: Age related changes of glucagon binding and activity in isolated rat hepatocytes. Int J Tissue React 8:367-371, 1986

51. Mooradian AD: The hepatic transcellular transport of 3,5,3′-triiodothyronine in aged rats. Biochim Biophys Acta 1054:1-7, 1990

52. Eaton RP, Conway M, Schade DS: Endogenous glucagon regulation in genetically hyperlipidaemic obese rats. Am J Physiol 230:1336-1341, 1976

53. Brown NF, Salter AM, Fears R, et al: Glucagon, cyclic AMP and

adrenaline stimulates the degradation of low density lipoprotein by cultured rat hepatocytes. Biochem J 262:425-429, 1989

54. Salter A, Fisher S, Brindley D: Interactions of triiodothyronine, insulin and dexamethasone on the binding of human LDL to rat hepatocytes in monolayer culture. Atherosclerosis 77:77-80, 1988

55. Kutt H, Herz J, Stanley K: Structure of the low density lipoprotein receptor related protein (LRP) promoter. Biochim Biophys Acta 1009:229-236, 1989

56. Descamps O, Bilheimer D, Herz J: Insulin stimulates receptor mediated uptake of apo E enriched lipoproteins and activated alpha 2 macroglobulin in adipocytes. J Biol Chem 268:974-981, 1993

57. Eriksson M, Berglund L, Rudling M, et al: Effects of estrogen on low density lipoprotein metabolism in males: Short-term and long-term studies during hormonal treatment of prostatic carcinoma. J Clin Invest 84:802-810, 1989

58. Bierman E, Albers J, Chait A: Effect of donor age on the binding and degradation of low density lipoproteins by cultured human arterial smooth muscle cells. J Gerontol 34:438-488, 1979